final

Chronic Inactivity

Male rats (Holtzman) weighing 255±7 grams at the beginning of each experimental period were used. Restraining cages (Figure 1) were adjusted weekly to maintain the relationship: cage volume in cc = 494.0 + 1.037(body weight in g). Control (unrestrained animals) was individually housed in 18 cm x 25 cm x 1 cm hanging wire cages. Food and water were allowed ad libitum, however, food was removed 24 hours prior to in vivo experiments to reduce the contents of the gastrointestinal tract.

Significantly more ($P<0.01$) deaths occurred in restrained (11 out of 450) than control animals (1 out of 450) and no deaths occurred after the 13th week. Growth curves (Figure 2) show a significant difference in mean body weight ($P<0.01$) at the end of one week between control and restrained groups which becomes more profound throughout the 25 week experimental period. Thus restrained animals do not grow as fast nor grow as large as unrestrained animals, but do continue to grow. As the length of time in the experimental housing increased the restrained aniamals became noticeably more docile, the musculature was flacid to the touch and the animals appeared weaker than control animals, i.e. were incapable of struggling.

No difference was observed between groups in either adrenal or thymus gland weights. While differences in hematocrit, fasting plasma glucose and leucocyte counts did occur between restrained and unrestrained groups, they were not consistent.

Glucose Absorption In Vivo

Plasma glucose changes during absorption were obtained from blood samples drawn from the tail into heparinized capillary tubes, before, immediately after, and at 10, 20, 30, and 45 minutes after test meal administration. After centrifuging and recording the packed cell volume, 25 microliters of the plasma were analyzed for glucose by the glucose oxidase method of Cawley et al. (2) without deproteinization.

In all in vivo experiments a one ml solution of 400 mg glucose in water was administered to each animal by stomach tube without previous anesthesia. Absorption was measured by the recovery and analysis of unabsorbed mutrient in the gastrointestinal tract at the end of a 45 minute absorption period. After sacrifice of the animals, the esophagus, pyloric sphincter and terminal ileum were ligated and the entire gstrointestinal tract removed. The stomach was separated from the intestine and the content of each rinsed out with five 10 ml

portions of warm saline and analyzed separately with Glucostat (Worthington Biochemical Corp.) after deproteinization with $ZnSO_4$-BaOH.

In one experiment changes in plasma glucose after test meal administration in 10 control and 10 restrained animals were recorded weekly for 25 weeks. Additional experiments of at least 10 control and 10 restrained animals each, were conducted after 5, 10, 15, 20 and 25 weeks of the experimental housing, in which, the unabsorbed contents of the gastrointestinal tract were analyzed as well as obtaining the changes in plasma glucose during absorption.

Curves of plasma glucose concentration during absorption after 5, 10, 15, 20 and 25 weeks of the experimental housing (Figure 3) show that the control groups remain essentially unchanged. Restrained groups over the same period show an upward trend. The regression lines (Figure 4) for the plasma glucose concentration at 30 minutes after the administration of the test meal to control and restrained rats illustrates this phenomenon. The control line shows no significant changes from the 5th to the 25th week while the regression line for the restrained animals increases linearly, has a significant slope ($P<0.01$)

and significant differences exist ($P<0.05$) between five week increments. Analysis of the unabsorbed contents of the gastrointestinal tract 45 min after test meal administration reveal no significant differences between restrained and control groups in the glucose recovered in the stomach, or in the total glucose removed from the tract (Table 1). Thus at the test meal concentration used, no difference in gastric empyting or in absorption of glucose could be demonstrated between groups after 5, 10, 15, 20 and 25 weeks.

Glucose Absorption In Vitro

The Crane and Wilson modification (3) of the everted intestinal sac method (Figure 5) was used. Unfasted animals were sacrificed, the intestine removed and rinsed with oxygenated mammalian Ringer solution. The first 10 cm from the pyloric sphincter and 10 cm from the center of the intestine (midgut) were everted, one end tied and the other end attached to a canula protruding through a rubber stopper. Incubation medium was Krebs-Ringer bicarbonate (5) with glucose-C^{14} plus carrier glucose added to make the final glucose concentration 555 mg/100 ml. The volume of the medium on the mucosal (outside) compartment of the intestinal segments were 35.0 ml. Enough of the medium was added through the

canula to the serosal (inside) compartment to raise the level of the liquid one to two cm above the level of the mucosal solution. The gas phase consisted of 95% O_2 - 5% CO_2, the incubation period was 45 min and the temperature 37 C.

At the end of the incubation period both serosal and mucosal solutions were brought up to a constant volume and samples of each counted in Bray's solution (1). The intestinal segments after weighing were digested in NCS-1 (a toluene solution of a quaternary ammonium hydroxide, Nuclear-Chicago Corp.) and counted. Calculation of the mg glucose was based on the quench corrected specific activity of the samples counted for a sufficient time to reduce counting error to less than 1.5%.

Liquid scintillation counting of glucose C^{14} recovered after incubation of the intestinal segments demonstrated a large increase in intestinal tissue concentration (1.2 to 3 times the concentration of the medium) as well as some increase in the serosal concentration, demonstrating active transport (Table 2). Water movement in the same direction occurs simultaneously with the movement of glucose from mucosal medium into the intestinal segment and on into the serosal medium. The final volume of fluid in the serosal compartment

always increased (from 0.05 to 0.36 ml above the intial volume).

Glucose oxidase analysis of final mucosal solutions failed to show any differences between control and restrained animals. Determination of the mean specific activities as well as total C-14 activity of mucosal solutions, serosal solutions, and the intestinal segments yielded the same lack of differences between treatments when the following were compared: (after the 5th week of the experimental housing), 27 control to 28 restrained duodenal segments and 17 control to 18 restrained midgut segments: (after the 40th week of the experimental housing), 10 control and 10 restrained midgut segments were compared. Thus no significant differences could be shown to exist in absorption in vitro of glucose between restrained animals after 5 or 40 weeks of the experimental housing.

Fructose Absorption In Vivo

A two ml test meal of fructose-C-14 plus fructose carrier (250 mg in water) was administered by gastric intubation to animals fasted 24 hours. Animals were sacrificed (cervical disjunction) 60 minutes after test meal administration. Ligatures were placed on the esophagus, pyloric sphincter and terminal ileum. The stomach, small and large intestine were then removed,

separated, and homogenized individually in a micro-Waring blender for five minutes. The homogenates were deproteinized with Somogyi reagents, filtered and a one ml sample of the filtrate prepared for C-14 analysis (4).

Radiochromatographs of the recovered material (deproteinized with trichloroacetic acid) show the C-14 to be confined to a single peak; therefore fructose absorbed was assumed to be fructose administered minus fructose recovered from the gastrointestinal tract at the end of the 60 minute absorption period (Table 3).

The regression line of control animals from the 5th to 25th week of experimental housing shows no change (Figure 6). Over the same period the regression line of the restrained group shows a significant slope ($P<0.01$). Furthermore, variance analysis demonstrates that the regression line for restrained animals differed significantly ($P<0.05$) from the unrestrained group.

Fructose Absorption In Vitro

Unfasted animals were sacrificed and the small intestine removed and rinsed free of its contents with oxygenated mammalian Ringer solution. The everted intestinal segment technique of Crane and Wilson were followed as described in the glucose studies. The initial concentration was always 200 mg/100 ml of the

medium.

When fructose-C-14 was initially present on both sides of an everted rat intestine, the mucosal fructose concentration was found to be greater than the serosal fructose concentration after 60 minutes incubation (Table 4). After incubation recovery of fructose (mg) from mucosal solution was shown to be considerably less than that initially added; however, there was no significant change in the amount of fructose present in the seosal solution. An increase in final serosal volume occurred as the result of water movement across the wall from the mucosal side. At the same time, the mucosal fluid volume was slightly decreased from the initial volume.

When fructose was placed only in the mucosal medium, there was a movement of fructose across the wall into the serosal solution with a concomitant movement of fluid in the same direction. Although no significant difference in fluid or fructose movement was observed between groups, more fructose was moved than when both compartments were initially filled with fructose medium.

The segment was also shown to permit sugar movement from serosal to mucosal solution when only the serosal medium contained fructose initially. However, the net fluid movement in the system always remained unidirectional, that is from mucosal to serosal solution.

Concentrations of both serosal and mucosal solutions did not differ significantly between control and restrained or between 5th and 25th weeks (Table 5). Movement of fluid from the serosal to mucosal solutions was shown to be greater in the 25th week than in the 5th week animals; however, the intestinal segments of both control and restrained animals contained from 31-52% less fructose per gram tissue fluid in the 25th week than comparable segments from animals in the 5th week.

Results of a study of 80 oven-dried intestinal segments from both groups of five week animals indicate that 80±1% of the wet weight is tissue fluid and that there is also no difference in per cent dry weight between groups (control versus restrained).

Phenylalanine Absorption In Vivo

In Vivo phenylalanine absorption studies were performed on rats chronically restrained from 5, 10, 15 and 25 weeks (Table 6). The quantity of phenylalanine absorbed in the restrained animals was significantly greater than that of the control experiments ($P<0.01$) at the 5th week period. During the following 10th and 15th weeks of restriction the restrained rats were also shown to absorb a greater amount of phenylalanine but this difference is not statistically significant.

A comparison of gastrointestinal absorption is expressed by the ratio of nutrient absorbed by restrained (R) over that of the control (C) rats or R/C. The R/C values decrease progressively with increase in length of immobilization and, at the 25th week, the amount by the unrestrained exceeds that of restrained animals although not to a significant degree.

Phenylalanine Absorption In Vitro

The amino acid uptake by the everted intestinal preparation after one hour incubation was expressed in terms of mg per 100 mg tissue wet weight as shown in Table 7. The intestinal segments of the 5th week restraint were demonstrated to absorb a slightly greater amount of phenylalanine than the control as similarly observed in the *in vivo* studies of the same period; however, the *in vitro* results failed to show a significant difference between groups.

Glycine Absorption In Vivo

In Table 8, the mean values for total quantity of absorbed glycine at different length of periods of restriction are shown. The difference in glycine absorption is not statistically significant throughout the entire 25 weeks of experimental housing; however, the value is always greater in the restrained state.

Oleic Acid Absorption In Vivo

Data obtained from oleic acid studies at 5, 15, 20 and 25 weeks are summarized in Table 9. A shift in absorption pattern with time by the restraint can be visualized from the R/C ratios. At the 5th week, the restrained rats absorbed approximately 1/3 of the amount taken up by the control rats, but this difference was progressively decreased until at the 25th week when the value for the restrained animals was slightly greater than that of the control animals.

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TABLE 1. Glucose Absorption *In Vivo*

Weeks*	Glucose Recovered† Stomach, mg		Glucose Absorbed,§ mg	
	Control	Restrained	Control	Restrained
5	53 ±15	72 ±16	337 ±15 (10)	322 ±17 (9)
10	27 ± 8	39 ±12	367 ±10 (10)	356 ±13 (10)
15	87 ±18	108 ±17	302 ±18 (10)	283 ±17 (10)
20	92 ±13	85 ±17	293 ±12 (15)	302 ±18 (12)
25	49 ± 8	45 ±10	347 ± 8 (18)	353 ±10 (20)

* Number of weeks of the experimental housing before *in vivo* tests.

† Glucose recovered in the stomach 45 min after 400 mg glucose test meal, ±S.E.

§ Glucose absorbed, mg = glucose administered minus glucose recovered in both the stomach and the intestine. Number of animals in parentheses.

TABLE 2. *In Vitro* Movement of Glucose at 5 Weeks

Treatment and Segment*	Mucosal Solution, mg	Serosal Solution,† mg	Intestine mg	Transported§ mg
Control-Duodenum				
Initial	200	3.26±0.02	0.0	
Final	194±1.2	5.05±1.13	4.00±0.65	5.84±1.06
Restrained-Duodenum				
Initial	200	3.29±0.14	0.0	
Final	194±1.2	4.48±1.00	4.62±0.78	5.81±1.07
Control-Midgut				
Initial	200	4.06±0.40	0.0	
Final	194±1.2	5.33±1.29	4.49±0.64	6.60±1.14
Restrained-Midgut				
Initial	200	3.59±0.18	0.0	
Final	191±2.2	7.62±1.91	4.90±0.62	8.93±2.19

* Type of everted intestinal segment used, Duodenal = first 10 cm from pyloric sphincter, Midgut = 10 cm segment from midgut. Initial = before incubation,

† Final = after 45 min incubation period.

Initial mg glucose varied on serosal side due to the addition of medium to this side until the fluid was 1 to 2 cm higher than the level of the mucosal medium.

§ Mg transported = final serosal mg minus initial serosal mg + final intestinal mg.

All values mean±S.E.

TABLE 3. Fructose Absorption In Vivo

Weeks †	Stomach mg Fructose Recovered		Fructose Absorbed, mg *	
	Control	Restrained	Control	Restrained
5	16.93±5.60	9.82±2.05	116.26±10.11 (9)	118.34± 8.77(10)
10	12.60±2.11	23.13±8.01	111.27± 8.78(10)	114.83± 7.80(10)
15	19.08±3.96	15.07±3.92	112.69± 5.25(10)	121.64± 6.23(10)
20	22.15±3.92	28.12±5.41	105.08± 4.23(10)	120.24± 3.47 (9)
25	16.34±4.41	16.54±8.64	132.00± 6.43 (9)	153.12±10.66 (9)

* Fructose absorbed = fructose administered minus amount recovered. Values represent means in mg±S.E. Number of animals in parentheses.

† Weeks of chronic immobilization.

TABLE 4. *In Vitro* Fructose Movement At 5 Weeks

		Mucosal, mg/100 ml	Serosal, mg/100 ml	Intestine, mg Recovered	Serosal Volume ml Increased
Control(11)	I	200.0	200.0		
	F	197.0±0.4	173.4±3.2	1.39±0.06	0.03±0.03
Restrained(11)	I	200.0	200.0		
	F	196.2±0.7	178.1±6.5	1.39±0.09	0.12±0.03
Control(12)	I	200.0	0.0		
	F	199.7±1.2	73.6±7.5	1.36±0.11	0.09±0.02
Restrained(11)	I	200.0	0.0		
	F	195.7±1.1	84.8±5.5	1.15±0.08	0.08±0.01
Control(6)	I	0.0	200.0		
	F	1.21±0.02	106.5±2.8	0.29±0.01	0.03±0.01
Restrained(6)	I	0.0	200.0		
	F	1.30±0.01	101.2±4.7	0.28±0.02	0.07±0.01

* I = before incubation. F = after 60 minute incubation period. Values represent means ±S.E. Number of animals in parentheses.

TABLE 5. *In Vitro* Fructose Movement At 25 Weeks

		Mucosal, mg/100 ml	Serosal, mg/100 ml	Intestine, mg Recovered	Serosal Volume ml Increased
Control(10)	I*	200.0	200.0		
	F	193.8±1.01	161.5±5.24	0.93±0.04	0.15±0.03
Restrained(10)	I	200.0	200.0		
	F	195.5±0.88	163.3±3.67	0.78±0.07	0.15±0.03
Control(8)	I	200.0	0.0		
	F	192.3±1.38	64.0±4.08	0.66±0.03	0.15±0.03
Restrained(8)	I	200.0	0.0		
	F	191.7±0.90	71.7±5.16	0.67±0.03	0.13±0.03

* I = before incubation. F = after 60 minute incubation period. Values represent means ±S.E. Number of animals in parentheses.

TABLE 6. Phenylalanine Absorption In Vivo

Weeks*	Phenylalanine Absorbed, mg ‡		R/C
	Control	Restrained	
5†	145.47± 7.97 (8)	179.14± 4.16(10)	1.231
10	161.17±18.04(10)	179.88± 5.30(10)	1.116
15	156.55± 7.20(10)	161.62± 9.06 (9)	1.032
25	175.79± 5.84 (9)	164.76± 9.62 (9)	0.937

* Weeks of chronic immbolization.

† $P<0.01$ (student "t" test).

‡ Values represent means in mg±S.E. Number of animals in parentheses.

TABLE 7. Phenylalanine Absorption In Vitro

Weeks*	Group	Phenylalanine Uptake+	Tissue Concentration‡	R/C
5	Control	0.242±0.03 (9)	338.3±4.12	1.264
	Restrained	0.306±0.07(10)	415.3±48.3	
25	Control	0.277±0.01 (8)	343.5±14.27	1.083
	Restrained	0.300±0.01 (7)	376.9±12.8	

* Weeks of chronic immobilization.

\+ mg/100 mg tissue wet weight. Number of animals in parentheses.

‡ mg/100 ml tissue fluid (80% wet weight).

TABLE 8. Glycine Absorption In Vivo

Weeks*	Glycine Absorbed, mg+		R/C
	Control	Restrained	
5	159.67± 6.49(19)	160.95± 7.72(20)	1.008
10	116.73±13.64(10)	129.10± 6.09(10)	1.106
15	91.94± 8.98 (9)	114.33± 8.07(10)	1.244
20	165.23± 6.09(18)	172.53± 7.51(18)	1.044
25	80.15±19.18(10)	83.93±18.06 (9)	1.047

* Weeks of chronic immobilization.

\+ Values represent means in mg±S.E. Number of animals in parentheses.

TABLE 9. Oleic Acid Absorption In Vivo

Weeks*	Oleic Acid Absorbed, µmole†		R/C
	Control	Restrained	
5	150±54(10)	53±76 (9)	0.353
15	148±14 (9)	108±39(10)	0.730
20	237±27 (8)	228±19 (6)	0.962
25	146±42 *8)	153±49(10)	1.048

* Weeks of chronic immobilization.

† Values represent means in µmoles±S.E. Number of animals in parentheses.

Figure 1. Control and restraining cages. The restraining cage volume changes linearly with body weight and body volume of animal.

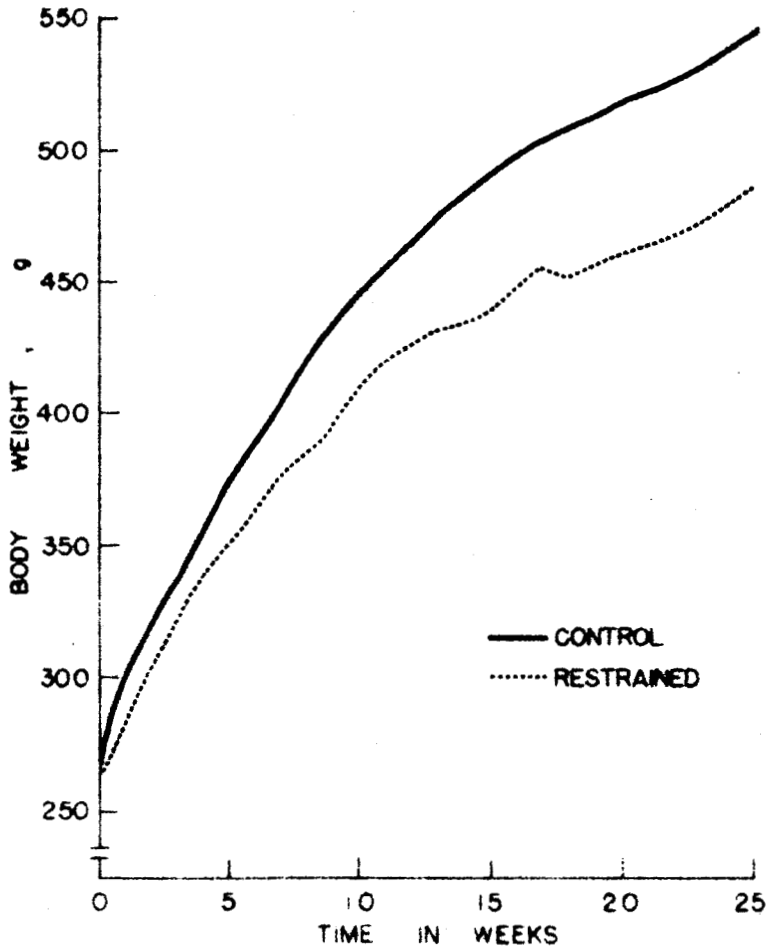


Figure 2. Growth curves as expressed by body weight in grams versus weeks of experimental housing.

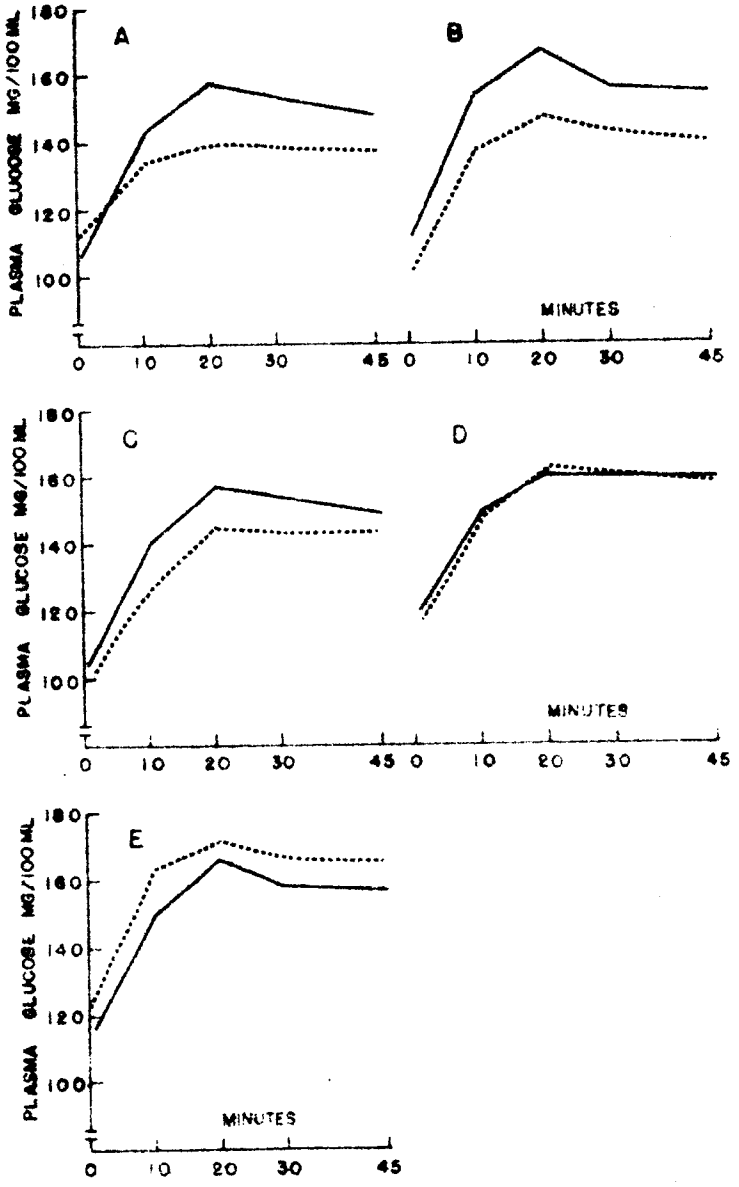


Figure 3. Plasma glucose concentration as a function of time. A, B, C, D and E represent 5, 10 15, 20 and 25 weeks of experimental housing respectively.

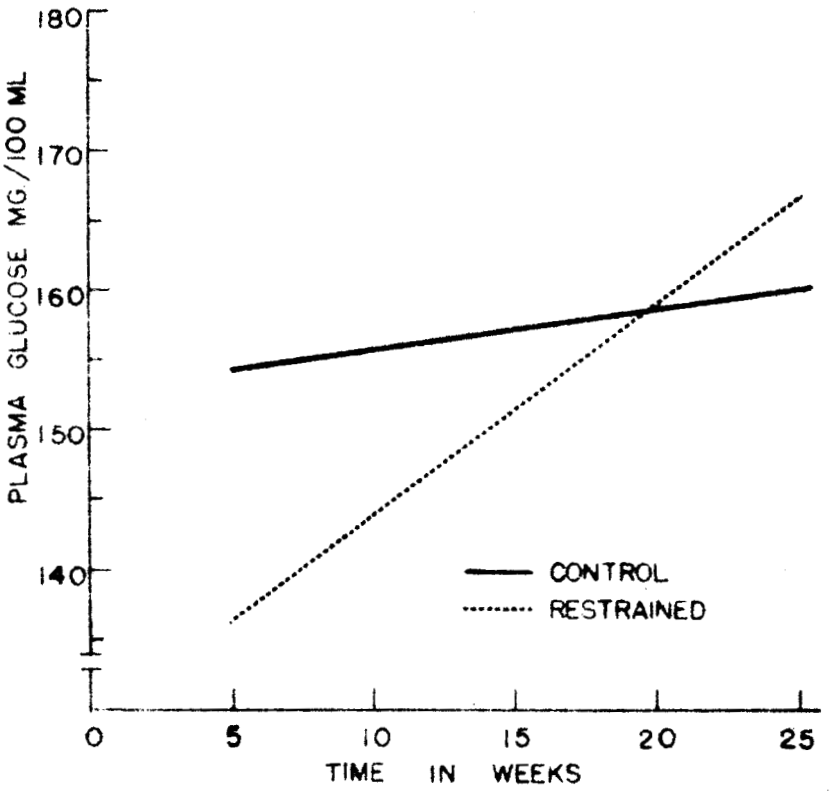
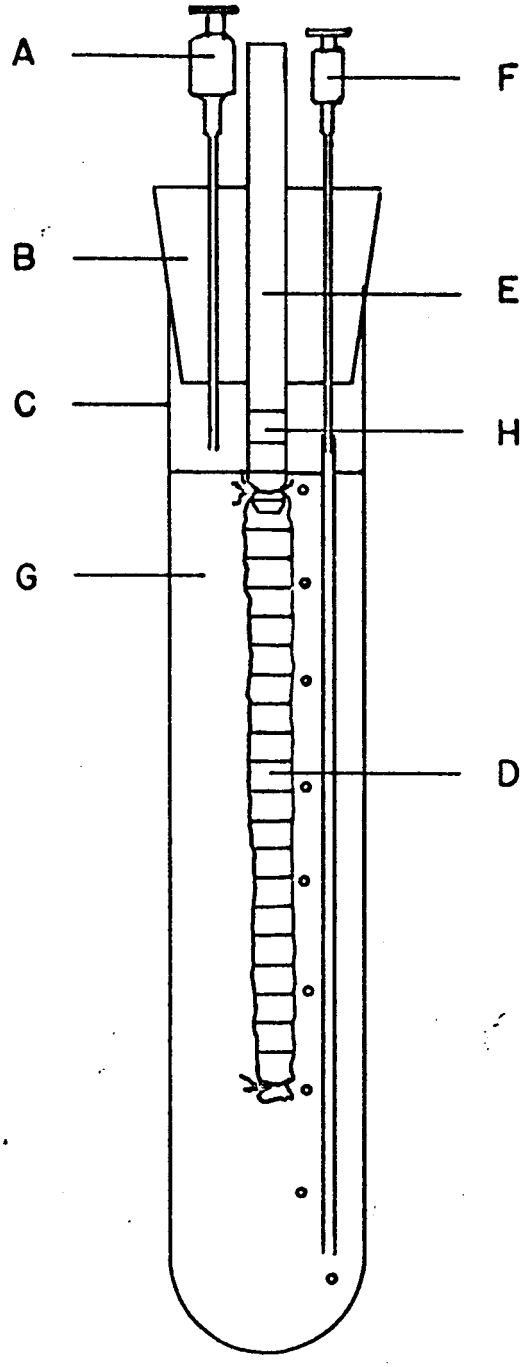


Figure 4. Regression lines showing the relationship between weeks of restraint and plasma glucose concentration at 30 minutes after test meal administration.

Figure 5. In Vitro intestinal preparation of Crane and Wilson.



A. 18-gauge needle
B. rubber stopper
C. 50 ml pyrex test tube
D. everted intestinal segment
E. glass canula
F. 22-gauge needle
G. mucosal bathing medium
H. serosal bathing medium

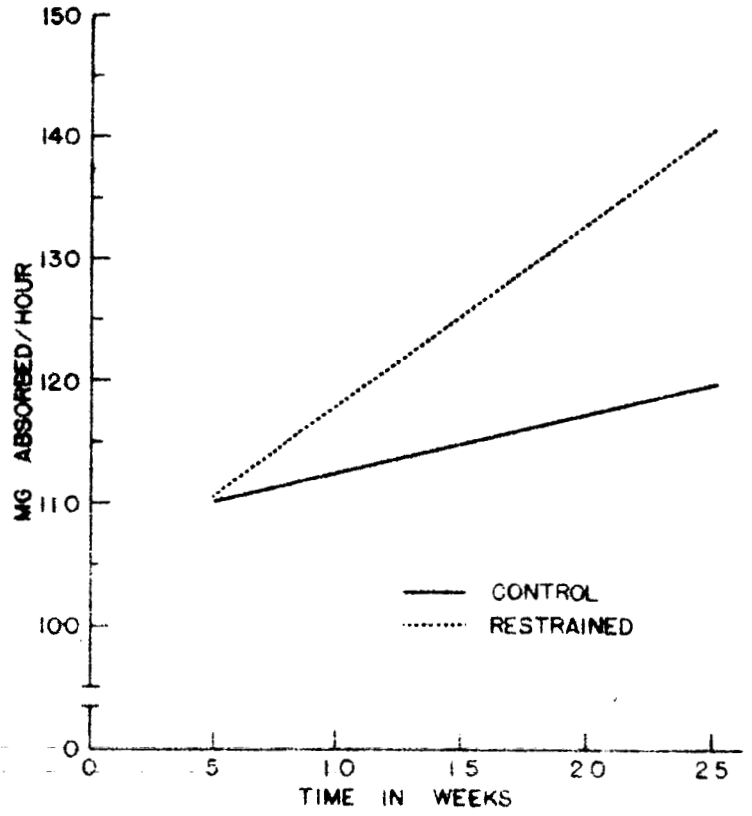


Figure 6. Regression lines, giving mg of fructose absorbed per hour <u>in vivo</u> as a function of weeks of experimental housing (5, 10, 15, 20 and 25).